Vol. LXVIII, No. 3 June, 1935

THE

BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE HEMOPOIETIC RESPONSE IN THE CATFISH, AMEIURUS NEBULOSUS, TO CHRONIC LEAD POISONING

ALDEN B. DAWSON

(*From the Biological Laboratories, Harvard University*)

In studies of the action of soluble salts of lead on fresh water fishes, Carpenter (1927, 1930) found that concentrations of lead nitrate as low as Pb 1: 3,000,000 proved lethal. The speed of lethal reaction was dependent upon the total quantity of metallic ions present as well as upon the actual concentrations used, and varied in inverse ratio to the size and weight of the fish employed. The most marked symptom was the formation of a film over the gills and skin of the fish by the interaction of the metallic ions with the surface mucus, causing death by suffocation. When insufficient lead was present the film was shed and complete recovery took place. Chemical analyses showed that no metallic ions had penetrated the body itself and Carpenter held that the action was purely an external process, chemical in type, but mechanical in its effect. In these studies the exposure to lead was of relatively short duration and probably little of the metal had entered the body, although the test (H_2S) used to detect the presence of lead in the ash residue of the body contents (Aub, Fairhall, Minot and Reznikoff, 1926) is not extremely delicate.

It seemed of interest accordingly to determine the relative efficiency of the mucus secreted by the exposed surfaces of fishes in binding the lead as an inert compound. In the light of other observations made on the effects of lead-poisoning in *Necturus* (Dawson, 1933*b*) it was felt that a biological test, the injurious effect on the circulating erythrocytes, might prove more satisfactory than the chemical detection of the metal in the tissues and body fluids of the animal.

Material and Methods

Young catfish 4 to 5 inches in length were used in this study. The animals were kept continuously in a bath composed of 20 cc. of 1 per cent lead acetate and 4 liters of tap water, which was changed every 48 hours. The experiments were begun in January and the experimental periods extended from 16 to 183 days. Samples of blood were drawn at regular intervals from a vein on the inner side of the operculum. The blood was studied in fresh preparation both by the Janus green-neutral red and the brilliant cresyl blue supravital techniques, and in dry smears stained by Wright's method. At the termination of the varying exposures to lead the animals and suitable controls were killed. The heart, liver, spleen, and mesonephros were removed, fixed in Helly-Zenker and stained with either hematoxylin and eosin or azur-eosin.

Effects of Lead Poisoning

Changes in the Peripheral Blood

In the catfish, practically all of the erythrocytes in the peripheral circulation are fully differentiated and basophilic or polychromatic cells are rare. In this respect they resemble those marine teleosts which are capable of removing dissolved oxygen from the water at a low oxygen tension (Dawson, 1933*a*). Exposure to lead produces no immediate or striking reaction within the blood stream. Intravascular phagocytosis of injured cells, such as occurred in *Necturus* (Dawson, 1930*a*), is almost entirely absent; the maximum number of active phagocytes ever observed in a study of any one supravital film (made with a circular cover-slip $\frac{3}{4}$ inch in diameter) being from 6 to 8. Injured cells appear smaller and become highly refractile; they are deeper in color, usually orange-yellow. Furthermore they are readily distorted, less elastic and consequently more fragile. In supravital preparations when slightly overstained with brilliant cresyl blue they appear green. With neutral red they are colored a deep red. Intracellular crystallization of hemoglobin, as in *Necturus* (Dawson, 1930*b*), is also readily induced by the slow drying of thick smears. Normally this does not occur although liberated hemoglobin crystallizes readily in this animal.

Following these early evidences of direct injury to the mature erythrocytes there is an increase in the number of polychromatic and basophilic cells, representing an exodus of incompletely differentiated erythrocytes from the erythropoietic loci, spleen, and mesonephros. At the end of thirty days a secondary anemia can be recognized and this increases in severity with the prolongation of the exposure to lead. Concomitant with the increased anemia, erythroblasts in varying stages of differentiation appear in the circulation and there is a marked increase in the number of small and large lymphocytes.

The cellular changes in the constitution of the blood, however, are not confined entirely to the cells of the erythrocytic series. Monocytes and the large granular eosinophiles are increased in number, but the most conspicuous change occurs in the numbers and relative proportions of thrombocytes and spindle cells (Fig. 9). These elements seem to be identical with the two types of cell distinguished by Jordan and Speidel (1930) in the blood of cyclostomes. The thrombocytes have a specific, fine reddish granulation with a homogeneous outer cytoplasm. The spindle cells are long and fusiform, frequently with their cytoplasmic processes recurved. They may possess granules similar to those of the typical thrombocytes. Both cells usually possess a similarly grooved nucleus. Jordan and Speidel, after reviewing the evidence on the identity of these elements, were unable to decide whether they were genetically related or independent. The typical spindle form in the catfish is not assumed in fresh preparations and the two types of cell are not readily distinguished previous to drying on the slide. It seems possible that the spindle-form is an atypical thrombocyte.

In normal blood the number of spindle cells is very small but after several weeks exposure to lead the number is greatly increased and practically equals that of the thrombocytes. This condition, when established, persisted throughout the experiments. Young thrombocytes are frequently present in considerable numbers. Some are relatively large and many give evidence of amitotic proliferation. The appearance of these atypical cells, probably all of the thrombocytic series, may be correlated with the activity of the endothelium of the heart, which will be described later. The evidence is not conclusive.

The occurrence of injured erythrocytes, the progressive anemia and the marked regenerative response in the peripheral blood indicated that large numbers of erythrocytes were being removed from the vascular channels although only a limited amount of intravascular phagocytosis could be detected. However, additional evidence of the degree of erythrocyte destruction was obtained from an examination of the liver, spleen, and mesonephros. In these organs there was a progressive storage of the hemoglobiniferous residue of degenerated red cells. In the heart no such accumulations were observed, but a marked proliferative response of the endothelium was obtained.

Changes in the Organs

In a recent study Mackmull and Michels (1932) reported on the sites and mode of storage of colloidal carbon injected into the peritoneal cavity of a teleost, the cunner. One hour after the injection, the vascular channels transported free carbon and carbon macrophages to such organs as the heart, gill, spleen, intestine, testes, ovary, and kidney. Extravascular migration of macrophages was most pronounced in the liver, spleen, kidney, and gonads.

The process of disposal of degenerate erythrocytes in the catfish parallels closely that of carbon storage in the cunner, especially in the liver, spleen, and mesonephros. Furthermore in the cunner, these organs normally contained collections of macrophages, the endogenous brown pigment of which was derived from degenerate red blood cells, and the migrating carbon macrophages displayed a selective orientation toward these areas of normal pigment deposit. However, in catfish, of the size used in this study, there are no such accumulations of stored pigment in these organs although a few isolated pigmented macrophages are occasionally found in their interstitial tissues. Accordingly the almost complete absence from the liver, spleen, and mesonephros of this pigment derived from dead erythrocytes furnishes a convenient base-line from which the amount of erythrocyte destruction may be determined by observing the progressive accumulation of pigment in the interstitial tissues by the macrophages.

Liver.—The liver of the catfish resembles that of the cunner (Mackmull and Michels, 1932), flounder, tautog, scup, minnow, and buffalo-fish (Jordan and Speidel, 1924) in that many of the veins are surrounded by sheaths of pancreatic tissue (Figs. 1 and 2). The pancreatic tissue is usually separated from the liver cells by a capillary space lined with reticulo-endothelium and frequently small foci of hemopoiesis may occur in the loose connective tissue in and about the intrahepatic pancreas. The progressive accumulation of pigment of erythrocytic origin within the liver is very marked (Figs. 3 and 4).

EXPLANATION OF PLATES

All figures are photomicrographs. Figures 1 and 3 were photographed under low power; Figs. 2, 4, 6, 7, and 8 under medium power; Figs. 5, 9, 10, 11, and 12 under oil immersion.

Plate I

1. A low power view of an area of the liver from a normal fish, showing the general appearance of the hepatic cells as well as of the intrahepatic pancreatic tissue which borders the veins of the liver.

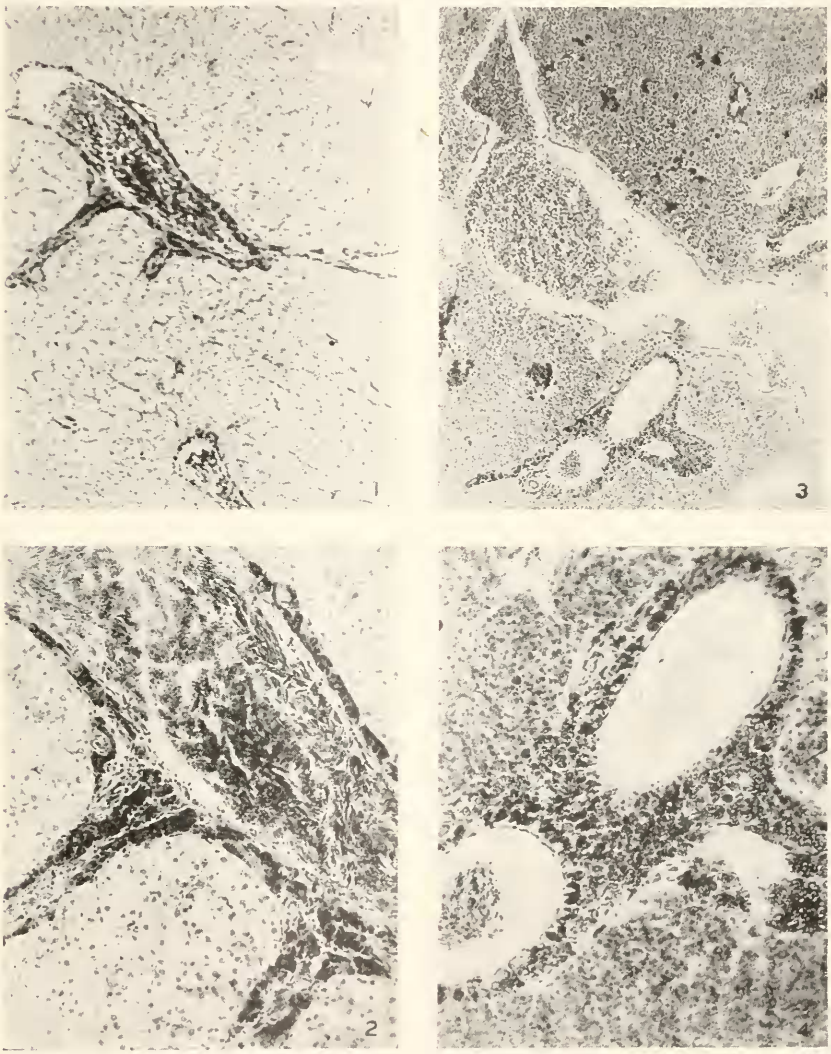
2. A portion of the above under greater magnification, showing the hepatic and pancreatic tissue in greater detail. Note the almost complete absence of brown and yellow granules (derived from disintegrated erythrocytes) in either the hepatic or interstitial cells.

3. A low power view of an area of the liver from a fish exposed to lead acetate for 183 days. The liver cells are small and dense and crowded with refractile yellow and brown granules. Masses of cells (macrophages) filled with similar pigment are scattered throughout the intertubular tissue. These cells are especially concentrated in and about the pancreatic epithelium.

4. A portion of the above under greater magnification, showing the concentration of pigment in the region of the pancreatic tissue.

The chief loci of storage are around the pancreatic cells although relatively large isolated groups of macrophages are also scattered throughout the organ between the hepatic cords. In addition to these sites of

Plate I



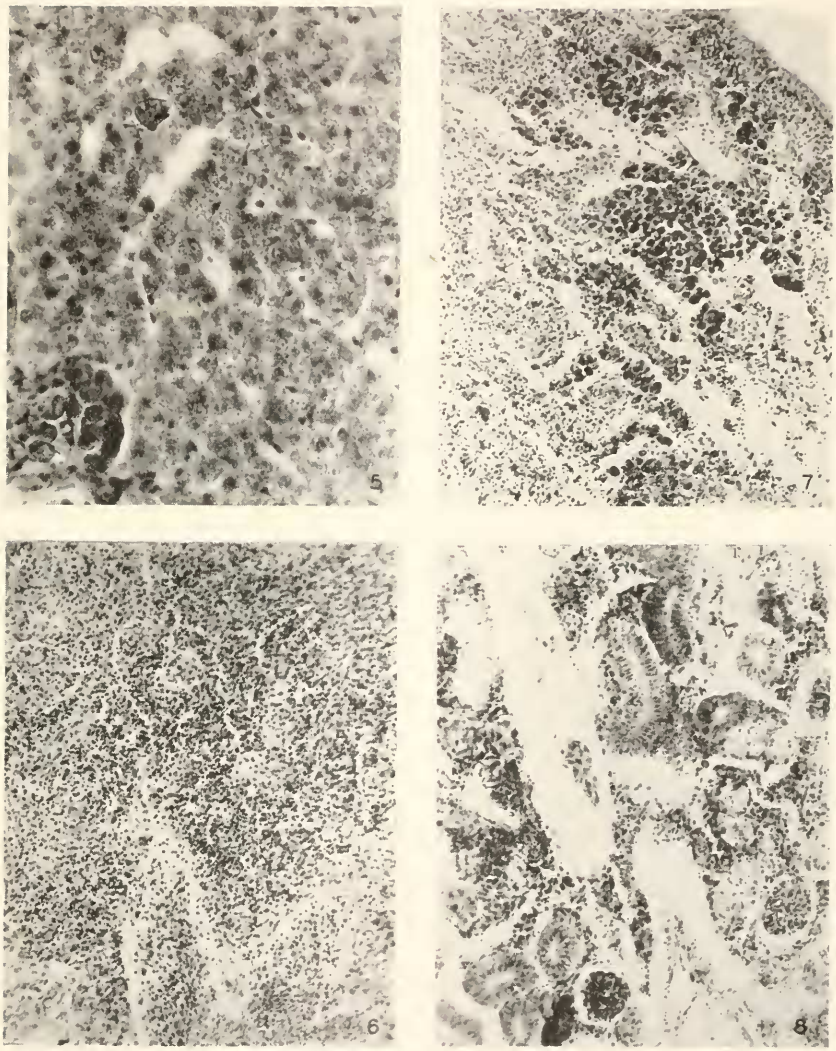
pigment storage, every liver cell is crowded with numerous brown refractive granules which appear similar to the granular contents of the macrophages (Fig. 5), but there is an almost complete absence of granular debris in the stellate cells of Kupffer, and in the endothelium of the veins associated with the pancreatic tissue. Only the lining of the capillaries in and about the pancreatic cells shows storage. Similar restriction of phagocytic activity of the vascular channels of the liver of the cunner was noted by Mackmull and Michels (1932) in carbon absorption. In fishes killed in late stages of lead poisoning the gall bladder is greatly dilated.

Spleen.—The general histological features of the spleen of fishes has been described by Yoffey (1929) and Mackmull and Michels (1932). The latter authors found that in the cunner the carbon macrophages and pigment macrophages migrated toward the splenic arterioles and occupied almost exclusively a periarterial position. As already pointed out in the normal young catfish, pigment macrophages are very rare, only a few isolated cells being present (Fig. 6). During lead poisoning the number progressively increases until the entire spleen is mottled with conspicuous yellow-brown masses (Fig. 7). Their distribution does not exactly coincide with that described for the cunner. Besides the definite concentration about the arterioles, there are equally large masses of pigment adjacent to the large splenic venules with which the arterioles are frequently closely associated. During lead poisoning the erythropoietic activity of the spleen is greatly reduced and few differentiating erythrocytes can be recognized in the pulp. The organ becomes smaller and paler.

Mesonephros.—The mesonephros in the teleosts is an important hemopoietic locus in which both erythrocytes and granulocytes are produced. The greater amount of hemopoietic activity is extravascular, occurring in the uniformly distributed loose intertubular connective tissue, but many of the peritubular capillaries show sinusoidal enlargements in which differentiating blood cells are also found. Pigment macrophages show no special groupings but local accumulations of such cells are scattered rather uniformly throughout the intertubular hemopoietic tissue. The progressive storage of this pigment during the exposure to lead is as evident in the mesonephros as in the liver and spleen but is never so extensive. No pigment was ever observed in the epithelium of the uriniferous tubules.

Heart.—Although pigment macrophages are commonly observed in the blood vessels of the spleen, liver, and mesonephros, they are not encountered either in the peripheral circulation or in the heart. They apparently move rapidly into the extravascular tissues of the organs and are not swept out into the general circulation. This is due largely to the fact that the degenerated erythrocytes are mostly phagocytosed by the attached reticulo-endothelial cells which later desquamate and enter the interstitial tissue.

PLATE II



5. A small area of hepatic tissue, showing the compact liver cells crowded with refractile granules. An interstitial mass of macrophages is seen in the lower, left corner.

6. An area from a normal spleen, showing the absence of pigmented macrophages.

7. An area from the spleen of an animal treated with lead acetate for 183 days. Note the massive infiltration of the splenic tissue by pigmented macrophages.

8. An area from the mesonephros from the same animal, showing the intertubular, extravascular concentration of pigmented macrophages. The mesonephric tubules are free of pigment.

In many of the lower vertebrates the endothelium and even the intermuscular connective tissue may display hemopoietic potencies. In the catfish the definitive lumen of the ventricle is small and the myocardium is made up of small muscle bundles separated by vascular channels, of the order and size of capillaries, which anastomose with one another to open eventually into the main ventricular cavity. The endothelium of these ramifying vascular channels is in intimate contact with the muscle fibers and in many instances does not appear to be sharply delimited from the intermuscular connective tissue.

In the cunner (Mackmull and Michels, 1932) both the endothelium of the minute intertrabecular vessels and the intermuscular connective tissue cells phagocytosed carbon. However, phagocytosis of injured erythrocytes was never observed in the heart of the catfish at any stage of lead poisoning and, as already noted, pigmented macrophages are also typically absent from the blood channels of this organ.

In the normal catfish the hemopoietic activity of the heart appears much less than in the cunner although small numbers of differentiating cells may occur in the capillary-like spaces of the myocardium. No lymphoid sheaths were observed. After several weeks of lead poisoning a marked proliferative response was obtained and relatively large clusters of cells were found on the surfaces of the muscle bundles (Fig. 10). The cells of such clusters are elongated and radiate from the site of proliferation into the lumina of the vascular channels. Their identity has not been definitely established, but it seems likely that they give rise to the atypical elongated and spindle cells described with the thrombocytes of the blood stream (Fig. 9). Presumably these clusters of cells arise by proliferation of the endothelium and there are some indications that they are secondarily invaded by cells of the intermuscular connective tissue.

PLATE III

9. Two areas from a blood smear of a fish exposed to lead acetate for 72 days, showing atypical elongated and spindle cells.

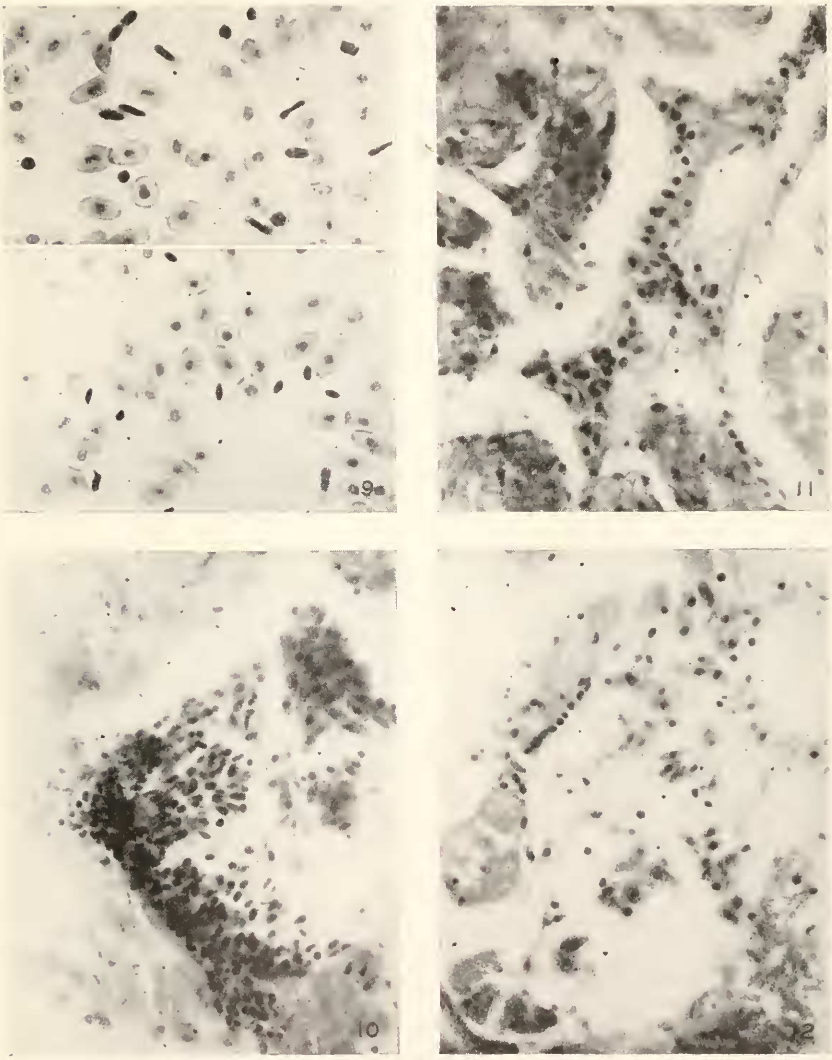
10. A region of proliferation from the surface of a muscular trabecula of the ventricle. The arrangement of cells resembles that in the margin of outgrowth of an explanted tissue. The elongated cells may give rise to the atypical cells provisionally classed with the thrombocytic series. The proliferating mass is apparently invaded by cells from connective tissue of the cardiac trabecula to form a reticular meshwork.

11. Another phase of the process of endothelial proliferation. The reticular meshwork is more obvious in this preparation. Small round cells and various stages of erythrocytic differentiation are present. Shrinkage has caused the separation of the tissue from the surface of the myocardial trabeculae.

12. This has been interpreted as a late stage of hemopoietic activity. The reticular meshes are distinct and almost empty but a few small round cells and erythrocytes are present. A row of small round cells remains attached to the surface of the trabecula.

In other regions of the heart less conspicuous areas of proliferation are also present. They apparently have the same relationship with the endothelium and connective tissues as the other proliferating cells,

Plate III



but in the latter case various stages of erythrocyte differentiation can be readily recognized (Figs. 11 and 12). The reticular-like cells are also more readily observed in these regions. The connective tissue and the endothelium of the ventricular wall of the catfish appear relatively undifferentiated, being essentially mesenchymal in nature and capable of hemopoietic activity although in this study they display no phagocytic activity toward the degenerate erythrocytes.

Discussion

These experiments with lead acetate furnish abundant evidence that teleosts such as the catfish slowly absorb lead when kept in solutions of this metal for relatively long periods. The surface mucus, while it may be effective in binding the lead for short periods (Carpenter, 1927, 1930), does not adequately protect the fish during long exposures. During exposures lasting for days and even weeks, it seems probable that lead in solution would eventually be also brought into contact with the epithelium of the digestive tract as well as with that of the gills and integument, and absorption may have been favored by this circumstance. In order to reduce this possibility to a minimum the animals were always removed to fresh tap water for feeding. However, there is no reason to believe that the mucin of the digestive tract would be less efficacious in binding the metallic ions than that of the gills and epidermis, but elimination of the mucous film could not be so readily accomplished in the digestive tract.

The observations on the peripheral blood demonstrate direct injury to the erythrocytes, followed by a mild regenerative response with the eventual development of a pronounced secondary anemia. The progressive storage of pigment, derived from phagocytosed erythrocytes, by the liver, spleen, and mesonephros also furnishes corroborative evidence of a widespread destruction of red blood cells. Since direct injury to the erythrocytes is generally the first and most important sign of lead poisoning in vertebrates, it seems safe to assume that the changes described in these experiments are the direct result of the absorption of lead.

The catfish apparently is less sensitive to lead than the fishes studied by Carpenter. Lead acetate rather than lead nitrate was used exclusively in my experiments, but no lethal effects were obtained although the concentration of lead was higher than that which proved lethal in Carpenter's series.

The changes in the peripheral blood of the catfish were comparable in many respects to those observed in an earlier study of lead poisoning in *Necturus*. The chief differences were in the absence of any considerable degree of phagocytic activity in the peripheral circulation and in the appearance in the circulation of large numbers of atypical cells, elongated and spindle forms, which may belong to the thrombocytic series.

The mode of accumulation and storage by the liver, spleen, and mesonephros of the pigment derived from the injured red blood cells follows closely that described in the elimination of particulate matter from the circulation of fishes. No storage occurred in the heart but a proliferative response to the destruction of the circulating elements was obtained. The erythropoietic potentialities of the cardiac endothelium of the lower vertebrates is well known and the literature is well summarized by Mackmull and Michels (1932). Indeed, in such forms as the herring (John, 1932) it may be the initial site of erythropoiesis. There are no red blood cells in the larval stages and they appear several months after hatching.

No evidence of direct injury to the thrombocytes was obtained and it is difficult to explain either the increase in number of the cells of this series or the atypical response of the cardiac endothelium in their production. The production of thrombocytes and erythrocytes usually takes place in the same organs or tissues and the two processes go on side by side. Furthermore the two types of cell are quite similar in their plan of organization. It appears possible that any factor which depresses or modifies erythropoiesis may tend to exert a similar influence on thrombopoiesis.

Summary

In the catfish, with prolonged exposure to a solution of lead acetate, definite evidence of absorption of lead was obtained. The surface mucus did not constitute an efficient barrier to the entrance of the metal as Carpenter (1927, 1930) found in more acute experiments with fishes in which a lethal reaction was frequently obtained.

The degree of destruction of the erythrocytes was used as a measure of the rate of the absorption of lead. Following the early evidences of injury to the blood cells, a mild regenerative response occurred, but eventually a pronounced secondary anemia was produced. Little phagocytosis of dead cells occurred in the peripheral circulation, but a progressive storage of the pigment derived from dead red blood cells was found in the interstitial tissues of the liver, spleen, and mesonephros. The hepatic cells also became crowded with pigment granules. No storage was observed in the heart. The pigment was accumulated chiefly in macrophages of local origin which, after ingesting erythrocytes, desquamated and migrated into the connective tissues.

Monocytes and eosinophiles were increased slightly in number in the blood stream, but the most striking change occurred in the numbers

of atypical thrombocytes, and spindle cells which may belong to the thrombocytic series.

The endothelium of the heart showed marked proliferative activity. In some regions there was localized differentiation of erythrocytes. In other regions the atypical elongated cells, just referred to, were formed in large radiating clusters on the surface of the ventricular trabeculae. No explanation of the latter response to lead poisoning can be given.

LITERATURE CITED

AUB, J. C., L. T. FAIRHALL, A. S. MINOT, AND P. REZNIKOFF, 1926. Lead Poisoning. Medicine Monographs, Vol. 7.

CARPENTER, K. E., 1927. The Lethal Action of Soluble Metallic Salts on Fishes. *Brit. Jour. Exper. Biol.*, **4**: 378.

CARPENTER, K. E., 1930. Further Researches on the Action of Metallic Salts on Fishes. *Jour. Exper. Zool.*, **56**: 407.

DAWSON, A. B., 1930 *a*. Intravascular Phagocytosis of Erythrocytes in Necturus Following Prolonged Immersion in Lead Acetate. *Anat. Rec.*, **45**: 345.

DAWSON, A. B., 1930 *b*. Changes in the Erythrocytes of Necturus Associated with the Intracellular Crystallization of Hemoglobin. *Anat. Rec.*, **46**: 161.

DAWSON, A. B., 1933 *a*. The Relative Numbers of Immature Erythrocytes in the Circulating Blood of several Species of Marine Fishes. *Biol. Bull.*, **64**: 33.

DAWSON, A. B., 1933 *b*. An Experimental Study of Hemopoiesis in Necturus; Effects of Lead Poisoning on Normal and Splenectomized Animals. *Jour. Morph.*, **55**: 349.

JOHN, C. C., 1932. The Origin of Erythrocytes in the Herring (Clupea harengus). *Proc. Roy. Soc.*, Ser. B, **110**: 112.

JORDAN, H. E., AND C. C. SPEIDEL, 1924. Studies on Lymphocytes. II. The origin, function, and fate of the lymphocytes in fishes. *Jour. Morph.*, **38**: 529.

JORDAN, H. E., AND C. C. SPEIDEL, 1930. Blood Formation in Cyclostomes. *Am. Jour. Anat.*, **46**: 355.

MACKMULL, G., AND N. A. MICHELS, 1932. Absorption of Colloidal Carbon from the Peritoneal Cavity in the Teleost, Tautogolabrus adspersus. *Am. Jour. Anat.*, **51**: 3.

YOFFEY, J. M., 1929. A Contribution to the Study of the Comparative Histology and Physiology of the Spleen, with Reference Chiefly to its Cellular Constituents. I. In fishes. *Jour. Anat.*, **63**: 314.